UNIVERSITI PUTRA MALAYSIA

TRANSESTERIFICATION OF REFINED, BLEACHED AND DEODORISED PALM OLEIN AND PALM OIL IN ORGANIC SOLVENTS BY IMMOBILISED RHIZOMUCOR MIEHEI LIPASE

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Chairman : Professor Dr. Hasanah Mohd. Ghazali

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The effects of medium's hydrophobicity and reaction temperatures on *Rhizomucor miehei* lipase-catalysed transesterification of refined, bleached and deodorised (RBD) palm olein and RBD palm oil were studied. Solvents with log *P* values ranging from -1.3-4.52 were chosen. The extent of transesterification was studied based on whether there was an increase in the triglyceride composition or if there were new triglycerides formed. Analyses on the triglyceride composition, melting properties and slip melting point of transesterified oils were carried out. Solvents with log *P* values between 2 and 4 ($2 \leq \log P < 4$) and more than 4 ($\log P \geq 4$) were found to give the highest degree of transesterification in palm olein and palm oil. Solvents such as hexane (log *P* 3.5) and isooctane (log *P* 4.52) were found to be particularly useful in both transesterification of palm olein and palm oil. No transesterification took place in solvents with log *P* less than 2 ($\log P < 2$). The effect of reaction temperatures was studied using isooctane and hexane. Transesterification rates were found to increase as the reaction temperatures increased indicating that the lipase was stable at high temperatures in organic

solvents. The highest degree of transesterification was found at 60°C. A lower degree of transesterification was found in palm oil transesterified at 30°C.

Two new triglycerides were synthesised at the end of the transesterification of palm olein, namely, tripalmitin (PPP) and distearoyl-oleoyl glycerol (SOS). No new triglycerides were formed in transesterified palm oil. Changes in the composition of several original triglycerides were also obtained in transesterified palm olein and palm oil. Melting properties of transesterified oil depended in general, on the chemical composition of the oils. Due to the complexity of the triglycerides, the heating curves were not easily interpretable. Formation of tripalmitin was found to be the cause of a new peak formed in the heating curve of transesterified palm olein. The slip melting point of palm olein and palm oil increased after transesterification. Exception was noted for transesterified palm oil at different reaction temperatures in isooctane and hexane incubated for longer incubation period where the slip melting point decreased.

The white particles formed after transesterification of palm olein at 40°C shaked for 6 hours at 200 revmin^{-1} were found to contain high melting glycerides, which were dominated by tripalmitin. The high melting glycerides were also high in palmitic acids with trace amount of oleic, stearic and linoleic acids. The heating curve of high melting glycerides consisted of a sharp and more prominent high-T peak and a small low-T peak. Due to the triglyceride composition of high melting glycerides, its slip melting point was found to be at 59°C.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

TRANSESTERIFIKASI MINYAK OLEIN DAN MINYAK KELAPA SAWIT DALAM PELARUT ORGANIK OLEH *RHIZOMUCOR MIEHEI* LIPASE TERSEKAT-GERAK

Oleh

HAZLINA BINTI AHAMAD ZAKERI

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Pengerusi : Profesor Dr. Hasanah Mohd. Ghazali

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Kesan hidrofobisiti medium dan suhu tindakbalas terhadap transesterifikasi minyak olein dan minyak kelapa sawit oleh *Rhizomucor miehei* lipase telah diselidiki. Pelarut dengan nilai log *P* di dalam lingkungan -1.3-4.52 dipilih. Transesterifikasi bergantung kepada samada terdapat kandungan trigliserida yang bertambah atau samada trigliserida baru terbentuk. Analisa dijalankan terhadap kandungan trigliserida, sifat lebur dan takat lebur minyak yang telah ditransesterifikasi. Pelarut dengan nilai log *P* antara 2 dan 4 ($2 \leq \log P < 4$) dan lebih dari 4 ($\log P \geq 4$) telah menghasilkan darjah transesterifikasi yang tertinggi dalam minyak olein dan minyak sawit. Pelarut seperti heksana (log *P* 3.5) dan isooktana (log *P* 4.52) telah dikenalpasti sebagai pelarut yang sesuai dalam transesterifikasi minyak olein dan minyak sawit. Tiada transesterifikasi berlaku dalam pelarut dengan nilai log *P* kurang dari 2 ($\log P < 2$). Kesan suhu tindakbalas dikaji menggunakan isooktana dan heksana. Kadar transesterifikasi bertambah apabila suhu tindakbalas ditingkatkan. Ini menunjukkan bahawa lipase stabil pada suhu tinggi dalam pelarut organik. Darjah transesterifikasi yang tertinggi ditemui

pada 60°C. Darjah transesterifikasi yang rendah diperolehi dalam transesterifikasi minyak sawit pada 30°C.

Dua trigliserida baru disintesis pada akhir transesterifikasi minyak olein iaitu tripalmitin (PPP) dan distearoyl-oleoyl gliserol (SOS). Tiada trigliserida baru terbentuk dalam transesterifikasi minyak sawit. Perubahan pada kandungan trigliserida sedia ada diperolehi selepas transesterifikasi minyak olein dan minyak sawit. Sifat lebur minyak yang telah ditransesterifikasi bergantung secara umumnya kepada kandungan kimia minyak tersebut. Oleh kerana trigliserida adalah kompleks, garis lengkung lebur sukar untuk ditafsirkan. Terbentuknya tripalmitin adalah penyebab kepada pembentukan puncak baru dalam garis lingkung lebur minyak olein yang telah ditransesterifikasi. Takat lebur minyak olein dan minyak sawit bertambah selepas transesterifikasi kecuali transesterifikasi minyak sawit pada suhu berbeza dalam isooktana dan heksana pada masa inkubasi yang panjang di mana ianya berkurangan.

Pepejal putih yang terbentuk selepas transesterifikasi minyak olein pada 40°C selama 6 jam pada 200 revmin^{-1} ditemui mengandungi gliserida lebur tinggi yang didominasi oleh tripalmitin. Gliserida ini juga mengandungi kandungan asid palmitik yang tinggi dengan sedikit kandungan asid oleik, stearik dan linoleik. Termogram gliserida ini terdiri dari satu puncak suhu tinggi yang tajam dan lebih menonjol dari puncak suhu rendah yang kecil. Akibat kandungan trigliserida lebur tinggi, takat lebur gliserida lebur tinggi adalah pada 59°C.

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I certify that an Examination Committee met on 13th June 2002 to conduct the final examination of Hazlina binti Ahamad Zakeri on her Master of Science thesis entitled "Transesterification of Refined, Bleached and Deodorised Palm Olein and Palm Oil in Organic Solvents by Immobilised *Rhizomucor miehei* Lipase" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science.

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I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

HAZLINA BINTI AHAMAD ZAKERI

Date : 26/7/2002

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CHAPTER 1

INTRODUCTION

The development of methods to improve the nutritional and functional properties of fats and oils is of great interest to food processors. The properties of a fat or an oil depend on its fatty acid composition and the commercial value of one fat or oil compared to another is based on their fatty acid structure (Macrae, 1985; Sheldon, 1996). By modifying the fatty acid composition of the fats and oils, their properties can also be modified to make them more suitable for specific applications (Macrae, 1985). Traditional methods of modification include blending natural fats and oils with different triglyceride compositions or by alkali-catalysed transesterification (Sheldon, 1996). Other methods are through fractional crystallisation, hydrogenation and interesterification (Macrae, 1985). The latter method is usually used to alter the properties of fats by randomly redistributing the fatty acids on the glyceride backbone to produce new fats that have different properties from the original fats.

Studies have been undertaken with a view to enhancing the commercial and nutritional quality of palm oil and its fractions by interesterification (Graille *et al.*, 1992). Palm oil is a unique oil such that it can be fractionated into palm olein and stearin, which differ in their chemical

and physical properties from each other and with palm oil itself (Goh, 1993). Thus, palm oil and its fractions can be exploited to give a wide range of products (Ong, 1989) either to be used in food applications (e.g. margarines and as frying medium) or in non-food applications (e.g. oleochemicals and soaps) (Pantzaris, 1987). Nowadays, these products are made possible by using enzymatic interesterification.

Interesterification reactions using enzymes as catalysts are more favoured than chemical catalysts. Enzymes in general are very efficient catalysts, environmentally acceptable, can react under mild conditions and are not bound to their natural role (Faber, 1995). The use of more specific enzyme catalysts for interesterification gives an additional level of flexibility in controlling the structure of modified fats. By exploiting the specificity of the enzymes, 'tailor-made' fats and oils can be produced. One type of enzyme that has this property is the 1,3-specific lipase (Weete, 1998; Willis and Marangoni, 1998). For instance, *Rhizomucor miehei* lipase which is 1,3-specific, has been used in the incorporation of capric acid into fish oil by an acidolysis process (Jennings *et al.*, 1999) and in the production of cocoa-butter equivalent from palm oil mid fraction in the chocolate and confectionery industries (Bloomer *et al.*, 1990).

Lipases or triacylglycerol hydrolases [EC 3.1.1.3] naturally catalyse the hydrolysis of lipids, which requires high water concentrations. However, at low water concentrations, lipase synthesises esters and participates in the interesterification reactions (Murukami, 1993). This behaviour shows that some enzymes are active in low water systems. In fact, enzymes such as cholesterol oxidase (Snijder-Lambers *et al.*, 1987), horseradish peroxidase (Lim and Yoo, 2000), hydroxynitrile lyases (Bauer *et al.*, 1999; Costes *et al.*, 1999), ligninases (Gorjup *et al.*, 1999) and proteases (Boonsithai and Phutrakul, 1996) are found to be active in hydrophobic

solvents. According to Laane *et al.* (1987), a technology that involves modification of the immediate surroundings of an enzyme is called solvent or medium engineering.

In such systems, the level of water greatly affects the activity and stability of the enzyme (Klibanov, 1989). It was found that enzymes are inactive in completely 'dry' system but the enzymatic activity is increased when the water level of hydration increased (Zaks and Klibanov, 1988b). Apart form this, solvent selection is also important to ensure that such stability and activity are retained (Klibanov, 1989). The polarity, hydrophobicity and other physicochemical properties of solvents (e.g. solvating ability and molecular geometry) are to be considered (Adlercreutz, 1996). The polarity or hydrophobicity of the solvents can be measured by the logarithm of the partition coefficient or log *P* value (Laane *et al.*, 1987; Adlercreutz, 1996).

Under this rule, hydrophobic or non-polar solvents are found to be suitable as reaction media. Hydrophilic or polar solvents on the other hand will result in the distortion of the enzyme's conformation, thus, inactivating the enzyme (Gorman and Dordick, 1992). Even though some activity can be detected in organic solvents, immersion of an enzyme in a non-aqueous medium causes disruption in the interaction that holds the enzyme's 3-D configuration, which will lead to denaturation and/or inactivation (Leach, 1993). Hence, enzymes are either used as lyophilised enzyme powders or immobilised enzymes on supports (Chaplin and Bucke, 1990; Adlercreutz, 1996). Furthermore, immobilised enzymes can be reused.

A survey of the literature found that not many studies were done on the use of various organic solvents as reaction medium for transesterification of palm olein or palm oil and on the use of suitable solvent for the production of high melting

glycerides (HMG) in transesterified palm olein (i.e. to produce superolein enzymatically). Therefore, the objectives of this study are:

1. To study the effect of various organic solvents with different log *P* values on lipase-catalysed transesterification of palm olein and palm oil.
2. To study the effect of temperature on lipase-catalysed transesterification of palm olein and palm oil.
3. To isolate high melting glycerides (HMG) of palm olein and to characterise the HMG and olein that are formed following transesterification of palm olein.

CHAPTER 2

LITERATURE REVIEW

Palm Oil

Palm oil is produced from the world's most productive oil-yielding plant, the oil palm or *Elaeis guineensis*. In Malaysia, the oil palm produced an average of 3.66 tonnes of oil per hectare of plantation area (Malaysian Palm Oil Board, 2002). Last year [2001] alone, Malaysia produced about 11.8 million tonnes (51%) and exported about 10.6 million tonnes (61%) of palm oil and its products in the world (Malaysian Palm Oil Board, 2002). Palm oil and its products are employed in numerous food and non-food applications. This is due to its chemical and physical characteristics that can be readily exploited to give a wide range of products. They are also safe and nutritious in the human diet and are highly competitive in price. Therefore, palm oil is the best substitute for other vegetable oils in many applications, as frying media and for making margarines, shortenings, soaps, oleochemicals and other products (Pantzaris, 1987; Patel *et al.*, 1999).

The oil palm is a unique plant because it produces two types of oil - palm oil from the flesh or mesocarp of the fruit and palm kernel oil (PKO) from the seed or kernel (Ong, 1989). The first step in palm oil processing is

at the mill where crude palm oil (CPO) is extracted from the fruit. CPO contains several non-glyceride impurities and has a dark yellow or red colour. To obtain a more saleable and edible products, CPO is further refined by neutralisation, bleaching and deodorisation processes to remove the unwanted impurities like free fatty acids, gums, odoriferous materials, pigments, trace metals and water to produce an end-product called refined, bleached and deodorised (RBD) palm oil (Salunkhe *et al.*, 1992). The RBD palm oil can be exported as such or fractionated into solid and liquid fractions known as palm stearin and palm olein, respectively (Ong, 1989), which differ in their physicochemical properties, fatty acid compositions and have different end uses.

Fractionation takes advantage of the fact that palm oil contains triglycerides with fatty acids of different chain length, unsaturation and melting points. There are essentially three types of fractionation - dry, solvent and detergent fractionations (Salunkhe *et al.*, 1992) where dry fractionation is commonly used because it allows a maximum yield of palm olein (Ong *et al.*, 1995). With double fractionation, one obtains low cloud point palm olein or so-called superolein and palm mid fraction (PMF) (Ong, 1989; Ong *et al.*, 1992). PMF, which is enriched with dipalmitoyl-oleoyl-glycerol (POP), in combination with a distearoyl-oleoyl-glycerol (SOS) rich fat can form a suitable cocoa butter equivalent (CBE) (Bloomer *et al.*, 1990; Chong *et al.*, 1992).

Due to different properties of palm stearin and olein, they can be easily manipulated by processes like hydrogenation and interesterification to widen further the applications of palm oil. However, unlike many other vegetable oils, palm oil is normally used in food processing without hydrogenation. Under the effect of a basic catalyst, the acyl groups of the triglycerides are distributed both intramolecularly and

intermolecularly (Sonntag, 1982). This provides the method of transferring saturated fatty acids to predominantly unsaturated fatty acids and *vice versa*. Interesterification leads to the production of a triglyceride mixture having a composition completely different from that of the original oil. Industrially, interesterification procedures are used to produce fats or oils that have important biological activities (Martinez *et al.*, 1999; Jennings and Akoh, 1999) and to produce cocoa butter substitutes (CBS) (Bloomer *et al.*, 1990; Chong *et al.*, 1992) from cheaper oils. Palm oil of more desirable melting point can be prepared by interesterification (Gunstone and Norris, 1983). With interesterification, palm oil can also produce palm olein and stearin (Salunkhe *et al.*, 1992) and palm olein interesterified with palm kernel oil can be used as vanaspati substitute (Majumdar and Bhattacharyya, 1988).

Composition of Palm Oil and Its Fractions

The physical characteristics such as melting point and solid fat contents (SFC) or hardness of oils and fats are determined by their chemical composition (i.e. fatty acid and triglycerides composition). For instance, the fact that palm oil is semi-solid at ambient temperature of 30°C is due to the presence of a mixture of triglycerides with different melting points and solubilities (Ong, 1989). The composition of fatty acids attached to a triglyceride molecule also determines the thermal behaviour of the triglyceride. The more saturated the fatty acids attached, the faster the triglyceride crystallises (Che Man *et al.*, 1999). The composition of palm oil (Table 1) is important for determining the authencity of palm oil; for modifying the characteristics through fractionation, hydrogenation, interesterification and hybridisation; for improving the quality and for extending its application. Palm oil contains a relatively simple fatty acid composition. It contains an equal amount of